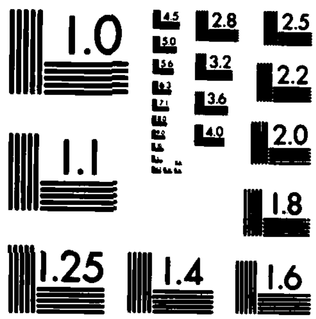
AD-A127 348 HPLC ANALYSIS OF SEX HMX TAX RDX AND TNT IN WASTEWATER 1/1
(U) ARMY MEDICAL BIOENGINEERING RESEARCH AND
DEVELOPMENT LAB FORT DETRICK MD E E BRUEGGEMANN JAN 83

UNCLASSIFIED USAMBRDL-TR-8206 F/G 19/1 NL

END
DATE
FILMED
5 83
DTIC


1.0
1.1
1.25
1.4
1.6
1.8
2.0
2.2
2.5
2.8
3.2
3.6
4.0
4.5
5.0
5.6

12

AD

TECHNICAL REPORT 8206

HPLC ANALYSIS OF SEX, HMX, TAX, RDX, AND TNT IN WASTEWATER

ERNST E. BRUEGGEMANN

US ARMY MEDICAL BIOENGINEERING RESEARCH and DEVELOPMENT LABORATORY
Fort Detrick
Frederick, MD 21701

JANUARY 1983

Approved for Public Release;
Distribution Unlimited

US ARMY MEDICAL RESEARCH and DEVELOPMENT COMMAND
Fort Detrick
Frederick, MD 21701

NOTICE

Disclaimer

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

Disposition

Destroy this report when it is no longer needed. Do not return it to the originator.

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

USAMBRDL REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER TECHNICAL REPORT 8206	2. GOVT ACCESSION NO. AD-A127 348	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) HPLC ANALYSIS OF SEX, HMX, TAX, RDX, AND TNT IN WASTEWATER		5. TYPE OF REPORT & PERIOD COVERED Technical Report Oct 81-Aug 82
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) ERNST E. BRUEGGEMANN		8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS US Army Medical Bioengineering Research and Development Laboratory, ATTN: SGRD-UBG Fort Detrick, Frederick, MD 21701		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 62720A 3E162720A835/AA/158
11. CONTROLLING OFFICE NAME AND ADDRESS US Army Medical Research and Development Command ATTN: SGRD-RMS Fort Detrick, Frederick, MD 21701		12. REPORT DATE January 1983
		13. NUMBER OF PAGES 22
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)		15. SECURITY CLASS. (of this report) UNCLASSIFIED
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

HMX
HPLC
RDX
SEX
TAX
TNT
Wastewater

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

A method for the separation and detection of 1-acetyloctahydro-3,5,7-trinitro-1,3,5,7-tetrazocine (SEX), 1-acetylhexahydro-3,5-dinitro-1,3,5-triazine (TAX), 1,3,5,7-tetranitro-1,3,5,7-tetraazacyclooctane (HMX), 1,3,5-trinitro-1,3,5-triazacyclohexane (RDX), and 2,4,6-trinitrotoluene by high performance liquid chromatography (HPLC) is described. A RAD-PAK A C_{18} column was used. A water/methanol mobile phase employing a gradient elution was used. The detector was set at 240 nm. With HPLC the separation of the

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

20. Abstract (continued)

different compounds was accomplished in <28 minutes. The detection limits were 0.2 µg/mL for SEX, HMX, TAX, RDX, and TNT. The method is simple, quick, and reproducible.

TABLE OF CONTENTS

INTRODUCTION....3

OBJECTIVE....3

MATERIALS....4

Equipment....4
Chemicals....4

METHODS....4

HPLC Conditions....4
Preparation of Stock and Standard Solutions....4
Concentration of Samples and Standards Using the SEP-PAK® C_{18} Cartridge....5

RESULTS AND DISCUSSION....5

REFERENCES....13

DISTRIBUTION LIST....21

APPENDIX

Appendix A. Precision and Accuracy Data....13

FIGURES

1. HPLC chromatogram of standard mixture of SEX, HMX, TAX, RDX, 2,4,6-TNT, and 1,3-DNB....9

2. HPLC chromatogram of wastewater influent containing SEX, HMX, TAX, RDX, TNT, and 1,3-DNB....10

3. HPLC chromatogram of wastewater effluent containing SEX, HMX, TAX, RDX, TNT, and 1,3-DNB....11

TABLES

1. R^2 Correlation Coefficient, Slope and Y-intercept for SEX, HMX, TAX, RDX, and TNT over a 4-day period....7

2. Percent Recovery of SEX, HMX, TAX, RDX, and TNT in Wastewater....8

3. Peak Area of Internal Standard over a 5-day Period....8

Use of trademarked name does not imply endorsement by the US Army, but is only to assist in identification of a specific product.

INTRODUCTION

The explosives 1-acetyloctahydro-3,5,7-trinitro-1,3,5,7-tetrazocine (SEX), 1-acetylhexahydro-3,5-dinitro-1,3,5-triazine (TAX), 1,3,5,7-tetranitro-1,3,5,7-tetraazacyclooctane (HMX), 1,3,5-trinitro-1,3,5-triazacyclohexane (RDX), and 2,4,6-trinitrotoluene (TNT) are being produced at several ammunition plants across the United States. When these plants are operating at full capacity, millions of gallons of wastewater (possibly containing these compounds) will be produced on a daily basis.

Environmental concern for military explosives has increased recently due to the mounting evidence that many, if not most, aromatic nitro derivatives possess biological properties detrimental to animals and humans.[1-4]

Since the wastewater effluents from these plants will probably contain explosives, it is necessary to develop a method for the trace analysis of these compounds. Thin-layer chromatography (TLC) of explosives has been reported in the literature.[5-7] This technique provides simple detection of explosives; however, quantitation suffers from the crudeness inherent in most quantitative TLC analyses. Gas chromatography (GC), because of its speed and sensitivity, received much attention as a method for the analysis of explosives. This technique, however, is limited due to the fact that many explosive compounds are thermally unstable.[8-10]

High performance liquid chromatography (HPLC) appears to be the method of choice for the analysis of explosives because HPLC can be conducted at ambient temperatures and without loss in efficiency of separation or speed of analysis. Also, time-consuming sample preparation in HPLC (under the proper conditions) is kept to a minimum. Direct injection of aqueous samples on a reverse phase liquid chromatographic system was first considered because of minimal sample preparation. However, because of possible column degradation by particulate matter and unknown (strongly retained) solutes, this idea was rejected. Also, the water to be analyzed was found to contain SEX, TAX, HMX, RDX, and TNT at levels too low for direct injection.

A sample preparation technique known as trace enrichment[11] was used with aqueous samples. A SEP-PAK® (Waters Assoc., Milford, MA) containing reverse phase (C_{18}) packing material was used to concentrate and clean up the samples prior to chromatographic analysis. The technique is simple, quick, and very reproducible.

OBJECTIVE

This analytical method was developed in support of research in wastewater treatment for the Holston Army Ammunition Plant, Kingsport, TN.

MATERIALS

EQUIPMENT

A Waters Liquid Chromatographic System® (Waters Assoc., Milford, MA) was employed throughout the study. The system consisted of the following components: two Model M6000A solvent delivery systems, Model 720 system controller, Model 730 data module, Model 450 UV-VIS variable wavelength detector, and Model U6K universal injector.

The column used was a flexible-walled cartridge (10 cm long x 8 mm I.D.) containing 10 µm reverse phase particles (C_{18}) under radial compression (Waters Assoc., Milford, MA). SEP-PAK® C_{18} cartridges (Waters Assoc., Milford, MA) were used in the trace enrichment technique.

CHEMICALS

1-acetyloctahydro-3,5,7-trinitro-1,3,5,7-tetrazocine (SEX), 1-acetylhexahydro-3,5-dinitro-1,3,5-triazine (TAX), 1,3,5,7-tetranitro-1,3,5,7-tetraazacyclooctane (HMX), 1,3,5-trinitro-1,3,5-triazacyclohexane (RDX), and 2,4,6-trinitrotoluene (TNT) were obtained from Holston Army Ammunition Plant (Kingsport, TN) and used without further purification. The internal standard 1,3 dinitrobenzene was synthesized and recrystallized in this Laboratory.[13] Solvents used for HPLC were of HPLC grade. Water used for HPLC was glass distilled.

METHODS

HPLC CONDITIONS

Separation of the explosives was achieved by using a reverse phase column (C_{18}) and a mobile phase of methanol and water. A linear gradient elution program was used in which the eluent was changed from 95% Pump A (25% $MeOH/H_2O$) to 50% Pump B (80% $MeOH/H_2O$) in 30 minutes at a flow rate of 1.7 mL/min and a pressure of 500 psi. The column effluent was monitored at 240 nm (0.1 absorbance units, full scale). The injection volume was always 100 µL.

PREPARATION OF STOCK AND STANDARD SOLUTIONS

A stock solution containing the five explosives was prepared by dissolving the solutes in acetone at a concentration of 100 mg/L. One milliliter of each stock solution was pipetted into 50, 100, 250, and 500 mL volumetric flasks. The contents of each flask were then diluted with glass-distilled water to yield standard solutions with concentrations of 2.0, 1.0, 0.4, and 0.2 ppm, respectively.

CONCENTRATION OF SAMPLES AND STANDARDS USING THE SEP-PAK® C_{18} CARTRIDGE

Before standards and samples are processed by this technique, it is essential that the cartridge be "activated" in the following manner. Two to four milliliters of methanol are passed through the cartridge to activate the packing material and remove organic impurities that might be on the cartridge. Following this, 15 mL of glass-distilled water are passed through the cartridge to displace the methanol. The cartridge is now ready for use.

One hundred microliters of a 200 ppm 1,3-dinitrobenzene solution (dissolved in methanol, internal standard) are added to 20 mL of each sample or standard solution. This is then passed through the SEP-PAK® cartridge at a flow rate of approximately 10 mL/min using a 50-mL glass syringe. Next, 10 mL of air are gently passed through the cartridge with a 10-mL glass syringe to displace the remaining aqueous solution from the packing material. Four milliliters of a 50% acetonitrile/water solution are then passed through the cartridge to remove the adsorbed explosives. This is followed by 10 mL of air. This eluent, including the air-displaced fraction, is collected in a glass screw-cap test tube.

The contents of the screw-cap test tubes are centrifuged at 2,000 rpm for 15 min. The supernatant liquids (both standards and samples) are transferred to individual sample vials. Samples and standards are now ready for analysis by HPLC.

RESULTS AND DISCUSSION

The ratios of peak areas of SEX, HMX, TAX, RDX, or TNT to peak area of the internal standard were plotted versus concentration of SEX, HMX, TAX, RDX, or TNT. By examining the R^2 correlation coefficients in Table 1, it can be seen that all the compounds show a linear relation over the concentration range analyzed ($R^2 > 0.995$). The lower detection limit was 100 ng.

Recovery studies were done with laboratory-spiked wastewater (Table 2). The peak areas of the SEP-PAK®-trapped materials were compared with standards having identical concentrations of the compounds examined. The recovery of SEX, HMX, TAX, RDX, and TNT was in the 70 to 76% range.

The peak area of the internal standard (1,3 dinitrobenzene) was followed over a 5-day period (Table 3). It appears that the SEP-PAK® cartridge trapped the internal standard from water with good reproducibility (S.D. ± 2%).

Figure 1 shows a chromatogram of a mixture of standard compounds (including internal standard). It can be seen that all the compounds were separated. Base-line resolution (R = 1.25)[12] was obtained for all compounds (including internal standard).

Figures 2 and 3 show typical chromatograms of a wastewater influent and effluent, respectively.* Both influent and effluent chromatograms show complete resolution of all the compounds with no interfering peaks present.

The method described is suitably precise, accurate, sensitive, and selective for the determination of SEX, HMX, TAX, RDX, and TNT in wastewater (see Appendix A).

* The influent was a synthetic wastewater with chemical oxygen demand (COD) of approximately 1,200 mg/L, mostly formaldehyde and formic acid, and was used to examine the treatability of Holston wastewater by a sequencing batch biological reactor. The effluent contained less than 100 mg/L COD.

TABLE 1. R^2 CORRELATION COEFFICIENT, SLOPE AND Y INTERCEPT FOR SEX, HMX, TAX, RDX, AND TNT OVER A 4-DAY PERIOD

Sample	Day 1	Day 2	Day 3	Day 4
SEX	slope = 0.125 Y intercept = 0.010 R^2 = 0.9995	slope = 0.130 Y intercept = 0.006 R^2 = 0.9997	slope = 0.120 Y intercept = 0.014 R^2 = 0.9997	slope = 0.132 Y intercept = -0.004 R^2 = 0.9997
HMX	slope = 0.631 Y intercept = -0.067 R^2 = 0.996	slope = 0.652 Y intercept = -0.011 R^2 = 0.999	slope = 0.678 Y intercept = -0.035 R^2 = 0.999	slope = 0.641 Y intercept = -0.043 R^2 = 0.999
TAX	slope = 0.117 Y intercept = 0.012 R^2 = 0.9991	slope = 0.119 Y intercept = 0.019 R^2 = 0.9859	slope = 0.112 Y intercept = 0.013 R^2 = 0.9994	slope = 0.105 Y intercept = 0.034 R^2 = 0.9925
RDX	slope = 0.322 Y intercept = 0.019 R^2 = 0.997	slope = 0.452 Y intercept = 0.007 R^2 = 0.999	slope = 0.372 Y intercept = -0.022 R^2 = 0.999	slope = 0.361 Y intercept = -0.011 R^2 = 0.999
TNT	slope = 0.641 Y intercept = 0.039 R^2 = 0.998	slope = 0.666 Y intercept = 0.037 R^2 = 0.999	slope = 0.548 Y intercept = 0.016 R^2 = 0.999	slope = 0.580 Y intercept = 0.035 R^2 = 0.999

TABLE 2. PERCENT RECOVERY OF SEX, HMX, TAX, RDX, AND TNT IN WASTEWATER

Sample	% Recovery
SEX	70
HMX	76
TAX	75
RDX	72
TNT	75

TABLE 3. PEAK AREA OF INTERNAL STANDARD OVER A 5-DAY PERIOD

Sample	Peak Area	Mean	S.D.
Day 1 (internal standard)	8,356,660		
Day 2 (internal standard)	8,362,131		
Day 3 (internal standard)	7,975,181		
Day 4 (internal standard)	8,198,366	8,170,093	± 197,107
Day 5 (internal standard)	7,958,129		(± 2%)

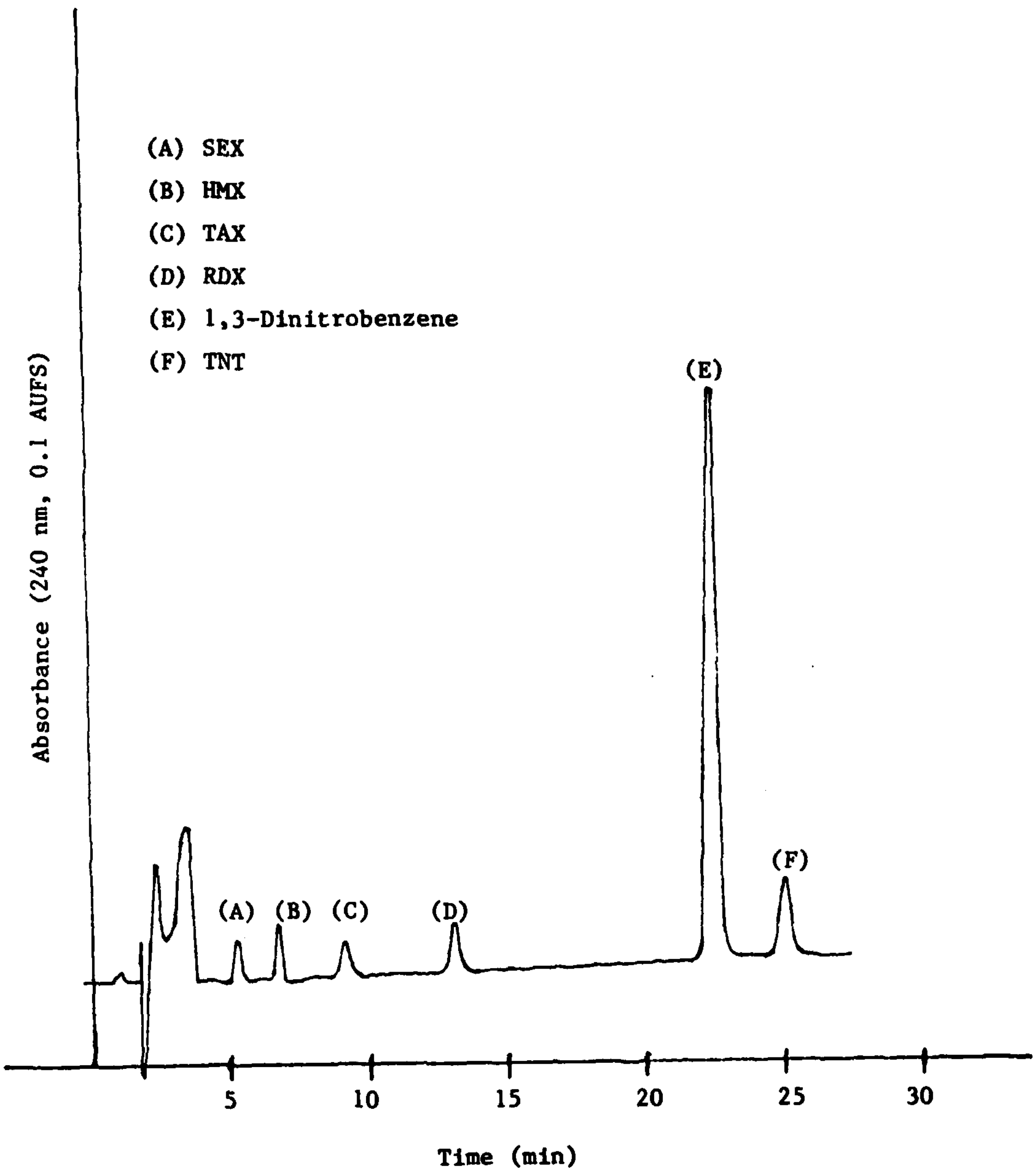


Figure 1. HPLC chromatogram of standard mixture of SEX, HMX, TAX, RDX, 2,4,6-TNT, and 1,3-DNB.

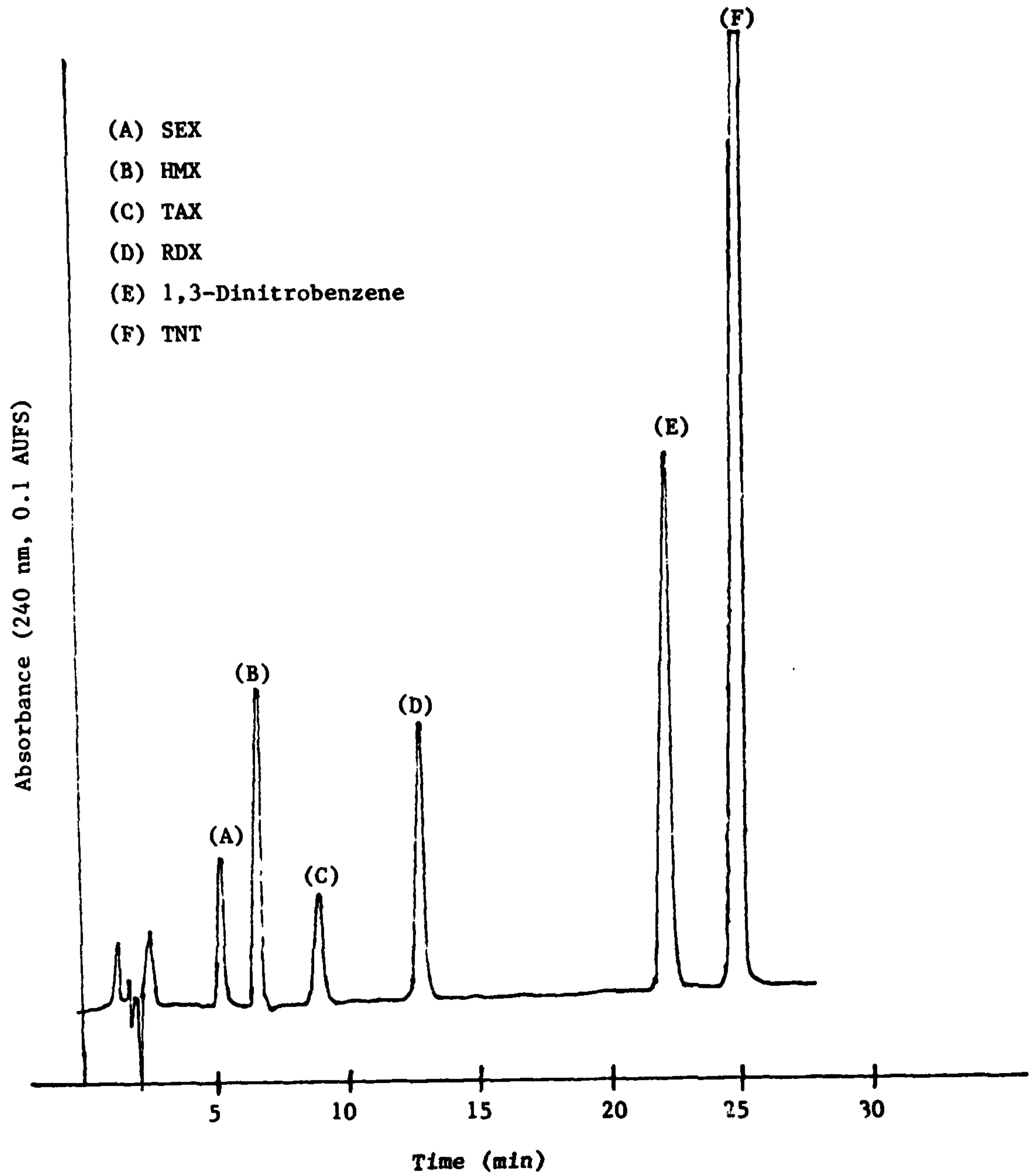


Figure 2. HPLC chromatogram of wastewater influent containing SEX, HMX, TAX, RDX, TNT, and 1,3-DNB.

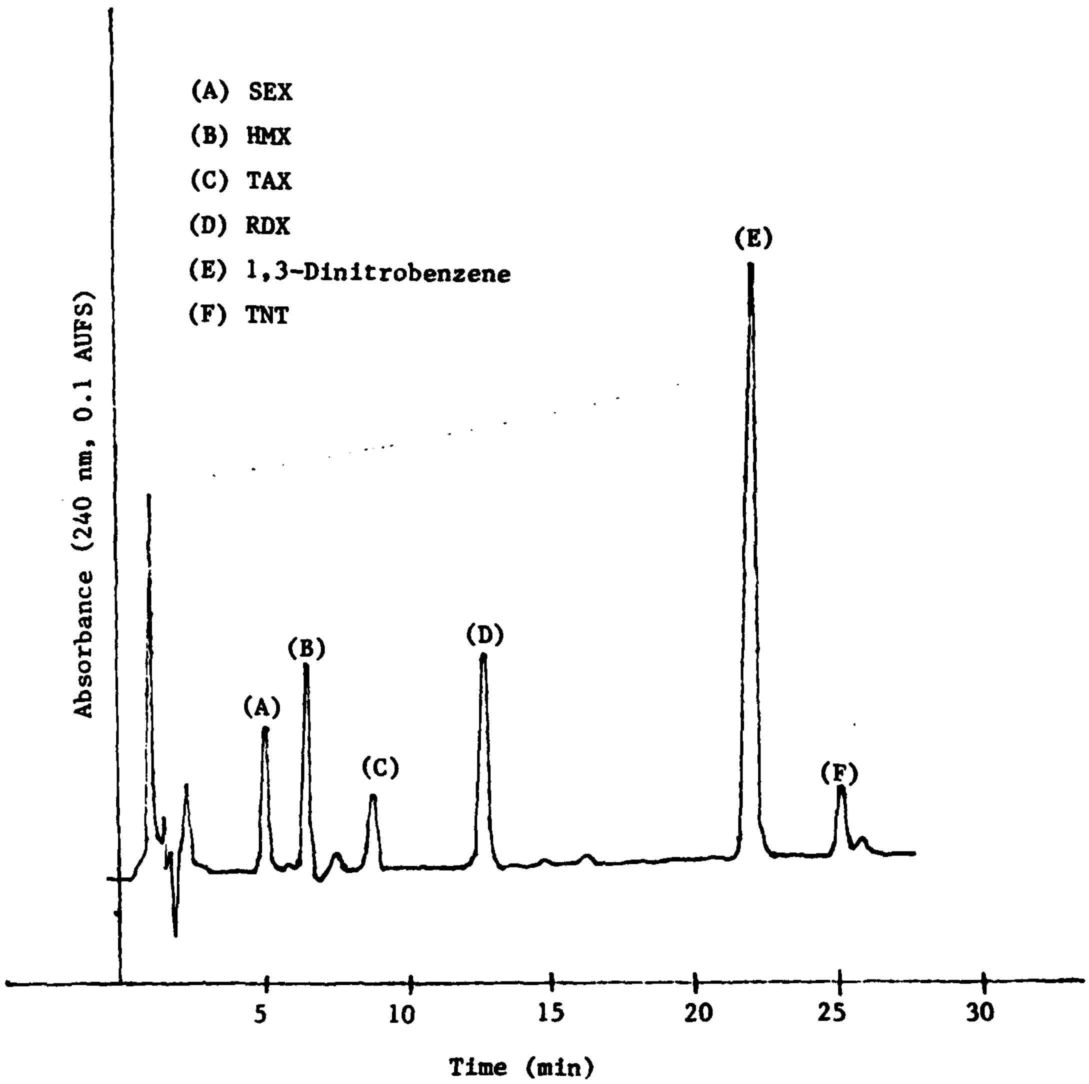


Figure 3. HPLC chromatogram of wastewater effluent containing SEX, HMX, TAX, RDX, TNT, and 1,3-DNB.

REFERENCES

1. Howard, P.H., J. Santodonato, J. Saxena, J. Malling, and D. Greninger. 1976. Investigation of Selected Potential Environmental Contaminants: Nitroaromatics. NTIS Rpt PB-275-078.

2. Won, W.D., L.H. Disalvo, and J. Ng. 1976. Toxicity and mutagenicity of 2,4,6-trinitrotoluene and its microbial metabolites. Appl. Env. Microbiol. 31:576.

3. Griswold, D.P., A.E. Casey, E.K. Weisburger, and J.H. Weisburger. 1968. The carcinogenicity of multiple intragastric doses of aromatic and heterocyclic nitro or amino derivatives in young female Sprague-Dawley rats. Cancer Research 28:924.

4. Goodall, C.M. and T.H. Kennedy. 1976. Carcinogenicity of dimethylnitramine in NZR rats and NZO mice. Cancer Letter. 1:295.

5. Yinon, J. 1977. Analysis of explosives. CRC Critical Reviews in Analytical Chemistry [CRC Press, Inc., Cleveland, (West Palm Beach, FL)]. Vol 1.

6. Hezel, U. 1978. Quantitative photometry of thin-layer chromatograms for research and routine analysis. Amer. Lab. 10(5):91.

7. Gurkin, M. and S. Cravitt. 1972. Quantitative and qualitative in situ analysis of thin-layer chromatograms. Amer. Lab. 4(1)

8. Camera, E. and D. Pravisani. 1964. Separation and analysis of alkylpolynitrates by gas chromatography. Anal. Chem. 36:2108.

9. Trowell, J.M. and M.C. Philpot. 1969. Gas chromatographic determination of plasticizers and stabilizers in composite modified double-base propellants. Anal. Chem. 41:166.

10. Norwitz, J.M. and J.B. Apatoff. 1971. Determination of dimethyl, diethyl and dibutyl phthalates in small arms double-base propellants by gas chromatography. J. Chromatogr. Sci. 9:682.

11. Saner, W.A. and J.R. Jadamec. 1979. Trace enrichment with hand-packed CO: PELL ODS guard columns and SEP-PAK C_{18} cartridges. Anal. Chem. 51(13):2180-2188.

12. Snyder, L.R. and J.J. Kirkland. 1979. Introduction to Modern Liquid Chromatography, pp. 34-49. John Wiley & Sons, Inc., New York.

13. Wertheim, E. 1948. A Laboratory Guide for Organic Chemistry, 3rd edition, p. 139. McGraw Hill, New York.

APPENDIX A

PRECISION AND ACCURACY DATA

TABLE A-1. ACCURACY DATA

Sample (day)	Actual Concentration (ppm)	Experimental Concentration (ppm)	% Recovery
		SEX	
1	0.59	0.66	111.86
2	0.59	0.59	100.00
3	0.59	0.67	113.56
4	0.59	0.61	103.39
		HMX	
1	0.63	0.62	98.41
2	0.63	0.60	95.24
3	0.63	0.66	104.76
4	0.63	0.57	90.48
		TAX	
1	0.64	0.88	137.50
2	0.64	0.70	109.38
3	0.64	0.66	103.13
4	0.64	0.59	92.19
		RDX	
1	0.67	0.69	102.99
2	0.67	0.70	104.48
3	0.67	0.69	102.99
4	0.67	0.67	100.00
		TNT	
1	0.61	0.55	90.16
2	0.61	0.58	95.08
3	0.61	0.57	93.44
4	0.61	0.58	95.08

TABLE A-2. PRECISION AT VARIOUS CONCENTRATION LEVELS, SEX

Sample (day)	ppm	Mean ppm	Standard Deviation	Relative Standard Deviation (%)
<u>0.244 ppm</u>				
1	0.25			
2	0.21			
3	0.22			
4	0.27	0.2375	±0.0275	11.58
<u>0.48 ppm</u>				
1	0.50			
2	0.51			
3	0.51			
4	0.45	0.4925	±0.0287	5.83
<u>1.19 ppm</u>				
1	1.15			
2	1.18			
3	1.18			
4	1.19	1.1750	±0.0173	1.47
<u>2.38 ppm</u>				
1	2.40			
2	2.38			
3	2.38			
4	2.38	2.3850	±0.0100	0.42

TABLE A-3. PRECISION AT VARIOUS CONCENTRATION LEVELS, HMX

Sample (day)	ppm	Mean ppm	Standard Deviation	Relative Standard Deviation (%)
<u>0.25 ppm</u>				
1	0.31			
2	0.26			
3	0.28			
4	0.29	0.2850	±0.0208	8.32
<u>0.50 ppm</u>				
1	0.50			
2	0.53			
3	0.54			
4	0.51	0.5200	±0.0183	3.52
<u>1.52 ppm</u>				
1	1.15			
2	1.18			
3	1.12			
4	1.17	1.1550	±0.0265	2.29
<u>2.51 ppm</u>				
1	2.56			
2	2.54			
3	2.56			
4	2.54	2.55	±0.0115	0.45

TABLE A-4. PRECISION AT VARIOUS CONCENTRATION LEVELS, TAX

Sample (day)	ppm	Mean ppm	Standard Deviation	Relative Standard Deviation (%)
<u>0.26 ppm</u>				
1	0.21			
2	0.18			
3	0.23			
4	0.18	0.2000	±0.0245	12.25
<u>0.51 ppm</u>				
1	0.56			
2	0.61			
3	0.56			
4	0.67	0.6000	±0.0523	10.25
<u>1.28 ppm</u>				
1	1.29			
2	1.46			
3	1.26			
4	1.16	1.2925	±0.1247	9.74
<u>2.55 ppm</u>				
1	2.54			
2	2.46			
3	2.55			
4	2.58	2.5325	±0.0512	2.02

TABLE A-5. PRECISION AT VARIOUS CONCENTRATION LEVELS, RDX

Sample (day)	ppm	Mean ppm	Standard Deviation	Relative Standard Deviation (%)
<u>0.27 ppm</u>				
1	0.34			
2	0.32			
3	0.31			
4	0.27	0.3100	±0.0294	9.48
<u>0.53 ppm</u>				
1	0.51			
2	0.54			
3	0.53			
4	0.56	0.5350	±0.0208	3.89
<u>1.33 ppm</u>				
1	1.25			
2	1.24			
3	1.26			
4	1.27	1.2550	±0.0129	1.03
<u>2.66 ppm</u>				
1	2.70			
2	2.70			
3	2.69			
4	2.68	2.6925	±0.0096	0.36

TABLE A-6. PRECISION AT VARIOUS CONCENTRATION LEVELS, TNT

Sample (day)	ppm	Mean ppm	Standard Deviation	Relative Standard Deviation
<u>0.24 ppm</u>				
1	0.26			
2	0.25			
3	0.26			
4	0.24	0.2525	±0.0096	3.80
<u>0.49 ppm</u>				
1	0.48			
2	0.49			
3	0.47			
4	0.49	0.4825	±0.0096	1.99
<u>1.22 ppm</u>				
1	1.20			
2	1.21			
3	1.21			
4	1.22	1.21	±0.0082	0.68
<u>2.44 ppm</u>				
1	2.45			
2	2.45			
3	2.45			
4	2.44	2.4475	±0.0050	0.20

DISTRIBUTION LIST

No. of Copies	
5	US Army Medical Research and Development Command ATTN: SGRD-RMS Fort Detrick, Frederick, MD 21701
12	Defense Technical Information Center (DTIC) ATTN: DTIC-DDA Cameron Station Alexandria, VA 22314
1	Commandant Academy of Health Sciences, US Army ATTN: AHS-COM Fort Sam Houston, TX 78234
2	Librarian US Army Medical Bioengineering Research and Development Laboratory ATTN: SGRD-UBZ-IL Fort Detrick, Frederick, MD 21701
1	Commander US Army Environmental Hygiene Agency ATTN: Librarian, Bldg E-2100 Aberdeen Proving Ground, MD 21010
1	Commander US Army Armament Research & Development Command ATTN: DRDAR-LCM-SA Dover, NJ 07801
1	Commander US Army Toxic and Hazardous Materials Agency ATTN: DRXTH-TE-D Aberdeen Proving Ground, MD 21010